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New Approaches in Correlative Studies of Biological Ultrastructure by High-Resolution Electron Microscopy

By

Professor H. Fernández-Morán, M.D., Ph.D.
Committee on Biophysics

THE UNIVERSITY OF CHICAGO

THE ENRICO FERMI INSTITUTE FOR NUCLEAR STUDIES

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By

Professor H. Fernandez-Moran, M.D., Ph.D.
Committee on Biophysics
University of Chicago
Chicago 37, Illinois

Paper presented at
ROYAL MICROSCOPICAL SOCIETY'S
Celebration of the "Tercentenary of the
Microscope in Living Biology"
April 9, 1963
Bethesda, Maryland

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*Supported by U.S. Atomic Energy Commission contract AT(30-1)-2278, by grants B-2460, C-3174, and NB-04267 from the National Institutes of Health, and by NASA grant NsG 441-63 from the National Aeronautics and Space Administration.

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Introduction

Three centuries of the microscope have immeasurably augmented man's dimensional span in the scale of living systems. Probing beyond the living cell into the confines of the molecular domain, microscopy has become the central unifying discipline of the natural sciences. In fact, the very essence of the living state has only recently been perceived as residing in the self-replication of certain macromolecules. This knowledge was gained essentially by enlightened fragmentation of the living state, experimentally resolved into its native constituents before reference could be made to the intact living cell. It thus borrowed more heavily from dissection of the mummy and the cadaver than from direct observation of the living body.

The light microscope extended man's direct range of vision nearly a thousand-fold, disclosing a whole new world for which there was at first no adequate reference system. It has taken three centuries to begin to make "sense", to correlate this new data with the available information and conceptual framework. Indeed, the predominant emphasis of the papers presented here on observations of the living state clearly indicate how much still remains to be learned from this direct visualization of biological processes at the microscopic level.

Supported by U.S. Atomic Energy Commission contract AT(30-1)-2278, by grants B-2460, C-3174, and NB-04267 from the National Institutes of Health, and by NASA grant NsG 441-63 from the National Aeronautics and Space Administration.

Yet, in many ways, this unprecedented flow of visual information conveyed by the microscope places the biologist in the strange predicament of an archaeologist who is being swamped by unearthing whole libraries and intact collections of records covering entire eras of ancient civilizations. The archaeologist is thus faced with the tantalizing dilemma of encountering an inconceivable wealth of systematized data which he unfortunately is unable to decipher, since the code is still largely unknown --awaiting the operational equivalent of a Rosetta Stone before it can be cracked.

This predicament is now compounded by the advent of high-resolution electron microscopy which further extends the capabilities for direct visualization of molecular structures.

Thus, even if we assume hypothetically that the formidable technical difficulties could eventually be overcome, fulfillment of the naive dream of directly observing a living cell by high-resolution microscopy would only overwhelm us with a torrent of bewilderingly complex and essentially "meaningless" or unmanageable information. Moreover, this possibliity of direct observation at the molecular level seems to be precluded by a fundamental limitation analogous to Bohr's complementarity principle. In analogy to the quantum state, any attempt to look at the detailed structure of a living biological system by direct observation would unavoidably seriously perturb or destroy it, since the requisite tools of observation would pour so much energy into the system that the native (low energy) condition would no longer hold.

However, this by no means excludes application of electron microscopy and its inherent preparation artifacts to the study of living biological systems. In fact, it can be demonstrated that electron microscopy is particularly suitable for the study of certain features of native biological systems, when adequately supplemented by the results of parallel biophysical and biochemical investigations (13,32,33,47).

This leads, then, to the interesting thesis that the supplementary analytical and "model" duplicative approach implied in correlative studies is not only operationally the most viable, but may eventually suggest novel and unexpected recombinations and syntheses. In this sense it may prove more feasible to "biosynthesize" key systems of living cells at the macromolecular level, than to analyse them completely by direct examination.

The ultimate challenge may well turn out to be elucidation not of living biological systems as we know them on earth, but instead of the viable modalities of the living state in space and time. And in this quest the microscope (in its broadest sense) will prove to be the prime analytical tool both for the direct observation and the controlled modification of preselected macromolecular regions of native biological systems.

In the following brief review, representative examples have been selected to illustrate characteristic features of new methodological approaches in correlative studies of native biological systems.

Fine Structure of the Nerve Myelin Sheath

Historically, the discovery of birefringence of nerves by Ehrenberg (6) over a century ago marks the first contribution of light microscopy to an understanding of the ultrastructure of a native "living" system. Subsequent polarization --optical studies of the nerve myelin sheath (46,49) indicated that it is composed of concentrically arranged submicroscopic protein or lipoprotein lamellae which alternate with lipid layers constituted of radially oriented lipid molecules. Further quantitative details of this highly ordered paracrystalline structure were then derived from the classic x-ray diffraction studies by Schmitt, Bear and Clark (48). The x-ray diffraction method offers a unique advantage of permitting examination of the intact nerve trunk in the living animal (48,49).

Moreover, the x-ray diffraction pattern represents an average of the main structural parameters of all the nerve fibers contained in the exposed nerve trunk. This technique thus provides precise information on important structural features of the nerve fibers under conditions of minimum perturbation which could never be matched by even the most refined light microscopy examination. Based on the available data, Schmitt et al (48,49) concluded that the living myelin sheath is of "smectic mixed-fluid crystalline nature and consists essentially of lipid-protein layers about 180 Å thick wrapped concentrically about the axon." The postulated layers and their exceptionally regular concentric arrangement in the myelin sheath were subsequently observed directly in the electron microscope (fig.1) (8,9,12). The fundamental radial repeating unit of 178 Å shown in the low angle x-ray diffraction pattern (fig. 1b) recorded from fresh frog sciatic nerve corresponds to the layer spacing of the myelin sheath with an average period of 130-140 Å, as seen directly in electron micrographs of osmium-fixed thin sections (fig. 1). Modifications introduced by the preparation techniques account for the difference of 20 to 40 Å between the two values.

By combined electron microscope and x-ray diffraction studies of controlled physical and chemical modifications of the myelin sheath, a general picture of the molecular arrangements in the radial units can be obtained (12,11,16). Such a combined approach has led to the detailed analysis of the preparation procedures, thus validating important findings and defining artifact sources.

The analysis of the fine structure of the myelin sheath can be regarded, in fact, as one of the best examples of the systematic application of complementary biophysical and biochemical methods. Moreover, since the myelin sheath derives from a multiply-folded Schwann-cell surface (45,12) it can also be regarded as a model system for the study of cell membrane structure in general. (15,20,25).

Electron Microscope and X-Ray Diffraction Studies of Crystalline Insect Virus Inclusions

The polyhedral inclusion bodies (2,40) formed by certain insect viruses in the nuclei of host cells are generally recognized as characteristic protein crystals (2) which contain the individual virus particles.

The macromolecular paracrystalline lattice of the protein component was first demonstrated by examinations of ultrathin sections of polyhedra with the electron microscope (40 n) thus confirming a suggestion which had been advanced previously on the basis of x-ray diffraction investigations (Kratky-2).

Further elucidation of the highly ordered fine structure of the polyhedral bodies appeared possible by the combined application of low-angle x-ray diffraction techniques and high-resolution electron microscopy. This correlation of the results of the two complementary methods had already contributed significantly to our knowledge of the ultrastructure of collagen, muscle, bone, and of the nerve myelin sheath (32,47,13). Moreover, the crystalline inclusions are favorable specimens to work with, and can be readily isolated in a pure form in large quantities.

Using material kindly provided by Dr. G.H.Bergold, preliminary studies have been carried out. (29). (The inclusion bodies, polyhedra and capsules were obtained from virus diseased lepidopterous larvae of Bombyx mori L. ,Lymantria monacha L., Cacoecia murinana, and from Laphygma frugiperda (J.E. Smith) appearing in Venezuela. All preparations were highly purified and stored in air-dried form.) The extraordinary degree of structural regularity (fig. 2a) characteristic of the crystalline inclusion bodies could be fully visualized free of distortions with improved ultrathin sectioning techniques using a diamond knife (10). It was possible to resolve additional details of the fine structure of the paracrystalline lattice of the polyhedra and of the other types of inclusions called capsules.

The preliminary data derived from the application of x-ray diffraction techniques to the study of untreated, or of viable modified and fixed virus inclusion bodies, are consistent with the main structural parameters of the paracrystalline lattice as revealed by electron microscopy. The information obtained by this collateral approach supplements in many ways the earlier observations of the regular molecular pattern at the surface of protein crystals. It also furnishes the basis for an investigation of the relationship between the inclusion body protein and the virus during intra-cellular development in the host.

Although these investigations have not been completed (and will be published in detail later) it was noted that the small angle x-ray diffraction pattern of these crystalline insect virus inclusion bodies exhibited characteristic spacings (fig.2b) which may be correlated with the fine structure revealed by electron microscopy. Here again it was possible to obtain by combination of the two techniques information on a system which is fully viable in terms of virus infectivity.

Correlation of Ultrastructure and Function in Mitochondrial Membranes

The high resolving power of the electron microscope enables us to visualize directly the individual cell membranes, which are only a few molecules in thickness (of the order 100 to 200 Å). These studies also confirmed earlier polarized light and x-ray diffraction investigations of fresh lamellar systems. It was revealed that a wide variety of related "lamellar systems" such as the nerve myelin sheath, photoreceptors (13,19), chloroplasts (4), and mitochondria, are in fact derivatives of multiply-folded cell membranes, disposed in highly ordered "paracrystalline" arrays. In living organisms, these specialized lamellar systems primarily implement specific energy-transduction functions with a remarkable degree of efficiency. Until recently, ultrastructural analysis had not progressed much beyond the stage of delineating the lipoprotein framework of the unit membranes. Technical

difficulties have stood in the way of obtaining precise information on the chemical composition of most of the lamellar systems.

Of all the specialized lamellar systems, mitochondria appear to be uniquely suited for successful implementation of correlative studies. As a result of this correlated biochemical and ultrastructural analysis, the mitochondrial membranes assume particular significance since we are dealing with one of the first lamellar systems in which the lipoprotein framework is gradually being invested with specific biochemical and enzymic detail (20,21).

Mitochondria are the "power plants" of the cell where the energy yielded by oxidation of food stuffs is harnessed in the form of the phosphate-bond energy of adenosine triphosphate (ATP), designated as "the universal intracellular carrier of chemical energy" (39). These mitochondrial transformations of coupling electron flow and citric acid cycle oxidations, which lead to the synthesis of ATP, involve the integrated interaction of at least seventy different enzymes and co-enzymes precisely arranged in a highly organized pattern.

The classic electron microscope studies of G. Palade (41,42) have shown that the rod-shaped mitochondria, approximately 1 to 10 microns long, are bounded by an outer double membrane with numerous transverse membrane infoldings, known as cristae. The mitochondria are circumscribed vesicles or membrane packages containing a semi-fluid matrix, and retain their most important enzymatic properties when carefully removed and isolated outside of the cell. Large-scale isolation has thus made these highly organized membranous organelles available for straight-forward chemical analysis. Properly isolated, intact mitochondria carry out the complete citric acid cycle and ancillary enzymatic functions, whereas the other two primary functions of electron transport and oxidative phosphorylation are localized in the organized mitochondrial membrane.

By careful serial dissociation of heart-muscle mitochondria, D. Green (33,34) and his associates were able to prepare well-

defined particles which contain the essentially complete electron transport chain. Improved preparation techniques for electron microscopy (17-23) have now been applied in systematic investigations of isolated mitochondria carried out jointly with D. Green and his associates at the University of Wisconsin.

In 1960-61, we detected a repeating particulate structure in the membranes of all types of mitochondria examined by electron microscopy with improved negative staining techniques. This entity, designated by us as the elementary particle (EP) (figures 3a, 3b) which had not been detected previously in standard osmium fixed thin section preparations, has since been observed by others (43). The particles could be seen disposed in highly regular array in the cristae and outer limiting membranes in preparations of both in situ and isolated mitochondria. These particles show characteristic dense substructure (fig. 4a) and consist of two recognizable parts: a polyhedral head (80 to 100 Å) and a cylindrical stem (about 50Å long and 20-40 Å wide)(figures 3a, 3b) that links the head piece to the central layer of the membranes. It is estimated that there are approximately 10,000 to 100,000 elementary particles per mitochondrion, depending on the size.

The elementary particles and associated membrane structures can be demonstrated reproducibly by a wide variety of techniques. This would indicate that the particles are actual components of the native mitochondrion. Recognition of this new repeating structural constituent led to the isolation and reconstitution of a particulate unit in homogeneous state. This particulate unit with a molecular weight of 1 to 1.4 million, and diameter of 140 to 160 Å, contains the complete electron transfer chain and closely resembles the native elementary particles.

The isolated or reconstituted particles (figures 4b,4c) appear to be a physical and functional aggregate of the four complexes known collectively to constitute the electron transfer system.

Extensive electron microscope and biochemical evidence now available is consistent with the assumption that the isolated or

reconstituted particles from beef heart mitochondria correspond essentially to the native elementary particles seen by electron microscopy (figures 4a,b,c). Correlated ultrastructural and biochemical studies carried out jointly with Green, Blair, and Oda (3,26) support the concept that this highly compact multi-enzyme unit is the ultimate seat of electron transfer in the respiratory chain.

Correlated research of mitochondrial membranes is of particular importance, because this lamellar system has been the first to yield concurrently to straight-forward biochemical and ultrastructural analysis at the same operational level of resolution. Instead of the relatively featureless, uniform membranes seen previously in osmium-fixed thin sections of mitochondria, it is now possible to identify the conspicuous arrays of elementary particles. Thousands of these compact respiratory enzyme assemblies are arranged in regular fashion on the hydrated lipoprotein framework of the mitochondrial membrane (model, Fig.5).

By extending these concepts to other lamellar systems, a general picture is obtained of paracrystalline lipoprotein layers containing compact "elementary particles" or "macromolecular assemblies" of specific enzymes, photopigments, "quantasomes" (4), or specialized electron transfer systems, all of which are organized in ordered three-dimensional patterns.

Improved preparation procedures which feature enhanced contrast and resolution obtained with combined negative-contrast and thin sectioning techniques, are yielding new information on the macromolecular organization within the plane of the layers of retinal rods (fig.6) outer segments, mitochondria, chloroplasts and other types of cell membrane derivatives.

Electron microscopy of negatively-stained solubilized lipids.- The water soluble pure mitochondrial phospholipids recently prepared by Fleischer and Klouwen (30,31) can now be examined directly by using improved negative staining techniques. This simple embedding of the lipid micelles in thin films of buffered phosphotungstate considerably reduces artifact sources, in contrast to the intricate staining effects which must be taken into account

when dealing with standard osmium-fixed and sectioned specimens.

In the lecithin micelles (figure 7) featuring periodic alternation of the hydrophobic and hydrophili groups of oriented lipid molecules, the buffered PTA reagent tends to localize preferentially at the available aqueous interfaces. Periodic dense lines (10 to 20 Å) separated by light bands (25-30 Å) with a regular spacing of 45 to 50 Å in the dried state, and of 60 to 80 Å in partly hydrated specimens, may therefore be interpreted primarily in terms of aqueous interfaces at the hydrophilic and hydrophobic regions of adjacent lecithin bimolecular leaflets. Here, as in the case of the mitochondrial membranes, successful application of the negative embedding techniques simplifies both the methodology and the interpretation, revealing new aspects of macromolecular organization in a more direct fashion(23-25).

Correlated electron microscopic and biochemical studies of the E. coli pyruvate dehydrogenation complex.

The E. coli pyruvate dehydrogenation complex has provided a unique opportunity to correlate functional properties as revealed by biochemical analysis with ultrastructure as revealed by electron microscopy (28,44). Recent methodological advances in high-resolution electron microscopy of biological systems have made it possible to visualize directly structural detail of the order of 6 to 8 Å under favorable conditions, hereby furnishing access to the molecular domain where structure and function are indissolubly blended. Electron microscopy has disclosed the substructure of membranes and of viruses (52), yielding results that can profitably be compared with x-ray data and biochemical information to deduce the actual arrangement of the protein subunits in certain spherical virus particles (5).

The picture of the structural organization of the pyruvate dehydrogenation complex which emerged from the biochemical studies of Dr. Lester J. Reed (44) is that of an organized mosaic of enzymes in which each of the component enzymes is uniquely located to permit efficient implementation of a consecutive reaction sequence. This picture has now been confirmed and extended by correlative electron microscope studies carried out in collaboration with the author. Moreover, through systematic application

of improved preparation techniques important structural details of the individual multienzyme complexes could be directly observed, hereby disclosing novel features of the molecular architecture (28).

Electron micrographs of the complex negatively stained with buffered phosphotungstate (figs. 8,9b) show various aspects of a regular polyhedral structure about 300 to 400 Å in diameter. Each of the rectangular or square structures corresponding to the multienzyme complexes embedded in different orientations features an orderly array of subunits, 60 to 90 Å in diameter. There appear to be four well-defined subunits in the central portion of the structural complex, and additional uniform subunits along each of the edges. These subunits are clearly depicted in preparations of the complex stained with uranyl acetate under special conditions (figs. 9c,d). Many of these subunits are similar in appearance and dimensions to those observed in electron micrographs of the isolated pyruvic carboxylase component for the complex. Various sub-fractions and controlled modifications of the complex were also studied by electron microscopy, which amounts to a form of controlled molecular dissection, since preselected components can be removed and the complex reconstituted in a stepwise fashion. These reproducible experiments have led to the tentative conclusion that the four central subunits correspond essentially to the lipoic reductase transacetylase aggregate. The molecules of pyruvic carboxylase and dihydrolipoic dehydrogenase are disposed in an orderly arrangement around this matrix aggregate.

A possible arrangement of the enzymic components of the complex is shown in a model (fig.9e) which is based largely on the results of electron microscopy.

Correlative studies of the native and reconstituted pyruvate dehydrogenation complex are of particular significance, because this multienzyme complex has been the first to yield concurrently to direct biochemical and ultrastructural analysis at an operational level of resolution adequate for elucidation of

its macromolecular organization. These studies will be reported in detail elsewhere (28).

The study of biological systems at liquid helium temperatures.

The shortcomings of our present preparation techniques are more acutely felt now that modern electron microscopes consistently achieve resolutions of the order of 5 to 10 Å, and are thus inherently capable of directly visualizing molecular structures in the size range of key enzymes and nucleoproteins. The development of adequate preparation methods is therefore a major problem which must be solved before high resolution electron microscopy can be more effectively applied in the study of biological systems during growth and function.

Since rapid freezing suspends all physiological activity, immobilizing and preserving labile tissue constituents, low-temperature techniques provide one of the most promising approaches toward reducing the complex preparation artifacts that impose serious limitations on all investigations of the living state. It therefore seems likely that significant advances in the study of life processes under conditions of minimum perturbation will depend to a large extent on further development of the unique potentialities inherent in the low-temperature domain. In turn, electron microscopy, x-ray diffraction and other physical techniques of molecular biology may well become the key analytical tools for critical evaluation of the basic parameters that determine optimum preservation of frozen biological systems.

Cooling to temperatures close to absolute zero does not appreciably impair critical life processes. This has now been amply demonstrated by the survival of a wide variety of living organisms, including bacteria, spermatozoa, and many other sensitive cells and tissues, which are first treated protectively with glycerol, then frozen with liquid nitrogen or liquid helium, and subsequently thawed in a controlled manner (16,17). By ultrarapid cooling to low enough temperatures it may be feasible to preserve the orginal position and relationship of the main organic and

inorganic constituents of cellular organization in tissues, including the predominant water component, the transient intermediates with unpaired electron spin which participate in enzymatic reactions and metabolic electron transfer, and other unstable chemical species generally referred to as free radicals.

However, in order to achieve this optimum preservation and effectively "fix" the highly reactive free radicals in biological systems, it would be necessary to work at temperatures of liquid helium(16,17,19,20), which are about 100°C lower than those currently obtained with isopentane-liquid nitrogen coolants.

Improved low-temperature preparation techniques for electron microscopy of biological tissues(16-23)have recently been developed which yield better morphological and histochemical preservation of lamellar systems and other cell components that do standard freeze drying of freeze-substitution methods. These "cryofixation" techniques(16,17) are based on rapid freezing of fresh or glycerinated tissues with liquid helium II at 1 to 2 degrees Kelvin, followed by freeze-substitution and embedding in plastics at low temperatures under conditions which minimize ice crystal formation, artificial osmotic gradients and extraction artifacts.

Beyond its applications in the investigation of serially arrested states of activity in biological systems, ultrarapid cooling with liquid helium II should prove useful in many other biochemical and biophysical studies(e.g. radiation biology)that require the attainment of very low temperatures. For example, the problem of establishing the ability of organisms to survive almost indefinitely at sufficiently low temperatures is of major importance. Although no detectable metabolic activity has been reported at liquid nitrogen temperatures, conditions verging upon unlimited conservation of viability can be expected only close to absolute zero at liquid helium temperatures, for which an incomparably greater reduction in metabolic rate has been calculated. In order to obtain accurate information on the ultrastructure of viable tissues at temperatures close to absolute zero, it would be necessary to perform electron microscopy and x-ray diffraction studies at liquid helium temperatures using

special instrumentation. It is only through the use of these refined analytical techniques that we may hope to establish whether "indefinite" preservation of biological systems is actually possible as we approach the realm of vanishing entropy at absolute zero.

General design concepts of cryo-electron microscope using superconducting electromagnetic lenses.-

As described in previous publications (16,17,24) the desirability of examining specimens at liquid helium temperatures led to the design of special low-temperature stages for electron microscopy. Our earlier work in this field has now been considerably extended as a result of recent major technical advances in the generation of high-field super-conducting magnets with solenoids of niobium-tin or of niobium-zirconium alloys (1).

Thus, by further development of the concepts embodied in our cryofixation techniques, it has been possible to design a new type of miniaturized high-resolution electron microscope totally immersed in liquid helium, which makes use of these completely stable super-conducting lenses, improved single-crystal pointed filaments and other distinctive features. These "cryo-electron microscopes", operating at temperatures of 1 to 4 degrees Kelvin, would embody the following significant features: (a) highly stable superconducting electromagnetic lenses, with very ripple-free magnetic fields of a persistent current in the optimum case; (b) operation in ultra-high vacuum and low temperatures resulting in decisive advantages of minimized specimen contamination, specimen damage and thermal noise; (c) optimum conditions for both low voltage (i.e. 1 to 10 kV) and high voltage electron microscopy. In addition, the use of high-efficiency image viewing (single-crystal fluorescent screens) and recording devices operating at optimum low temperatures would make it possible to use high-speed cinematography and stroboscopic recording (e.g. obtained through pulsed T-F emission from pointed filaments) for attainment of high temporal resolution combined with high spatial resolution.

In principle, such a cryo-electron microscope would also be an ideal device for controlled application of electron microbeams (50 Å to 500 Å diameter) of precisely defined intensity and duration for ultraminiaturization, storage of information, and in general for controlled irradiation and manipulation of hydrated biological systems at the molecular level under conditions of minimum perturbation. The described combination of optimized instrumental design parameters operative under conditions of minimized specimen perturbation represents one of the most promising coherent experimental approaches towards attainment of the theoretical resolution limit (about 2 Å) in direct examination of organic and biological structures.

At present an instrument of the type shown in figure 10 is being developed at our laboratories in the University of Chicago, as part of a comprehensive research program in the field of low-temperature electron microscopy.

In conclusion, one may be tempted to predict that the next centennial will see the microscope come to the fore to assume a key operational role which could well transcend anything we can imagine at present. In particular, we may find it intimately linked to a broader concept of life operative in extreme physical domains of low temperatures, high pressures, high radiation fields, etc., which are currently excluded from consideration.

ACKNOWLEDGEMENTS The author gratefully acknowledges stimulating discussions with Professor Francis O. Schmitt, Neurosciences Research Program and Massachusetts Institute of Technology, with Professor William H. Sweet, Massachusetts General Hospital, Professor William Bloom, University of Chicago, and with Dr. David E. Green, University of Wisconsin. Sincere thanks are also due to Frederick B. Merk and Charles Hough for their valuable technical assistance, and to Patricia Ricci and Joyce Bartels for their kind help in preparing the manuscript.

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Professor H. Fernandez-Moran, M.D., Ph.D., Committee on Biophysics, University of Chicago, Chicago, Illinois

ROYAL MICROSCOPICAL SOCIETY "Tercentenary of the Microscope in Living Biology" April 9, 1963

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TEXT TO ILLUSTRATIONS

"New Approaches in Correlative Studies of Biological Ultrastructure by High-Resolution Electron Microscopy"

by Professor H. Fernández-Morán, Committee on Biophysics
University of Chicago, Chicago, Illinois

ROYAL MICROSCOPICAL SOCIETY "Tercentenary of the Microscope in Living Biology", April 9, 1963

Figure 1 (a) High-resolution electron micrograph of myelin-sheath segment from transverse section of frog sciatic nerve demonstrating concentric array of dense and intermediate layers. Magnification: 350,000 x.

(b) Low-angle x-ray diffraction pattern of fresh rat sciatic nerve recorded with Finean camera. This pattern features a fundamental period of 178 Å, with characteristic alteration of the intensities of the even and odd orders.

Figure 2 (a) High-resolution electron micrograph of thin section of a polyhedral body of L. monacha showing virus particle embedded in regular paracrystalline lattice. Magnification: 510,000 x.

(b) Small-angle x-ray diffraction pattern of these crystalline insect virus inclusion bodies exhibiting characteristic spacings which may be correlated with the fine structure revealed by electron microscopy.

Figure 3 (a),(b) Electron micrograph of negatively stained mitochondrial membranes showing regular arrangement of the "Elementary Particles" (EP). These particles show characteristic dense substructure and consist of two recognizable parts, a polyhedral head (80 to 100 Å) and a cylindrical stem (about 50Å long and 40 Å wide) that links the head piece to the central layer of the membranes. Magnification: 650,000 x.

Figure 4 HIGH RESOLUTION ELECTRON MICROGRAPHS OF NEGATIVELY STAINED:
(1) Membrane segment (cristae) from isolated beef heart mitochondria with paired arrays of "elementary particles" "EP" (showing dense substructure) attached to central membrane layer;
(2) Isolated particles that contain complete electron transfer chain;
(3) Reconstituted electron transfer particles.
Magnification: 1,300,000 x.

Figure 5 Model of Mitochondrion based on recent electron microscope studies showing regular arrangement of repeating particulate structures (Elementary Particle attached

to mitochondrial membranes. It is estimated that there are about 10,000 to 100,000 elementary particles per mitochondrion, depending on size. Correlated ultrastructural and biochemical studies carried out jointly with Dr. D.E. Green and his associates at the University of Wisconsin support the concept that these highly compact multienzyme units (EP) are the ultimate seat of electron transfer in the respiratory chain. Scale of model given by particle diameters of ca. 100 Å.

Figure 6 Negative-stained thin section of frog retinal rod outer segment illustrating the enhanced contrast and resolution obtained with combined negative-contrast and thin-sectioning techniques. Magnification: 500,000x.

Figure 7 Electron micrograph of Lecithin micelles embedded in thin PTA film showing typical periodic arrangement of the bimolecular lipid layers. Magnification: 1,000,000x.

Figure 8 High-resolution electron micrograph of E. coli pyruvate dehydrogenation complex (PDC) prepared by Prof. Lester J. Reed. Complex negatively stained with 1% phosphotungstate (pH7.4) using microdroplet cross-spraying technique. Magnification: 650,000x.

Figure 9 HIGH RESOLUTION ELECTRON MICROGRAPHS OF E. COLI PYRUVATE DEHYDROGENATION COMPLEX:

(a) Reconstituted PDC negatively-stained with phosphotungstate; Magnification: 1,000,000x
(b) Native PDC complex showing four (4) control subunits and associated structures; Magnification: 1,000,000x.
(c),(d) Complex positively stained with 2% uranyl acetate. Subunit structures shown in different orientations. Magnification: 1,000,000x.
(e) Model of E. coli pyruvate dehydrogenation complex based on the results of correlated electron microscopic and biochemical studies carried out in collaboration with Prof. Lester J. Reed.

Figure 10 Sketch of Cryo-electron microscope with superconducting electromagnetic lenses.

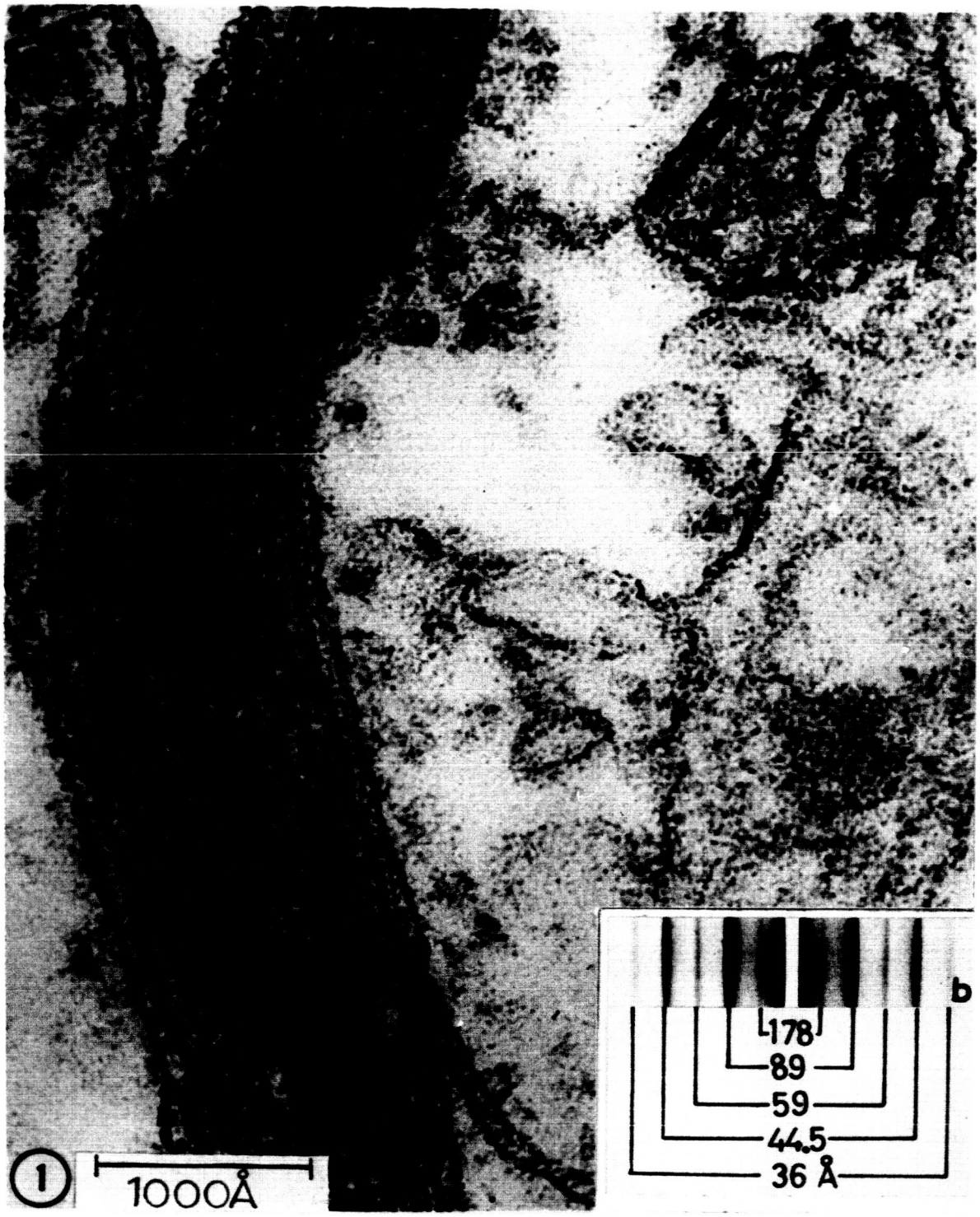
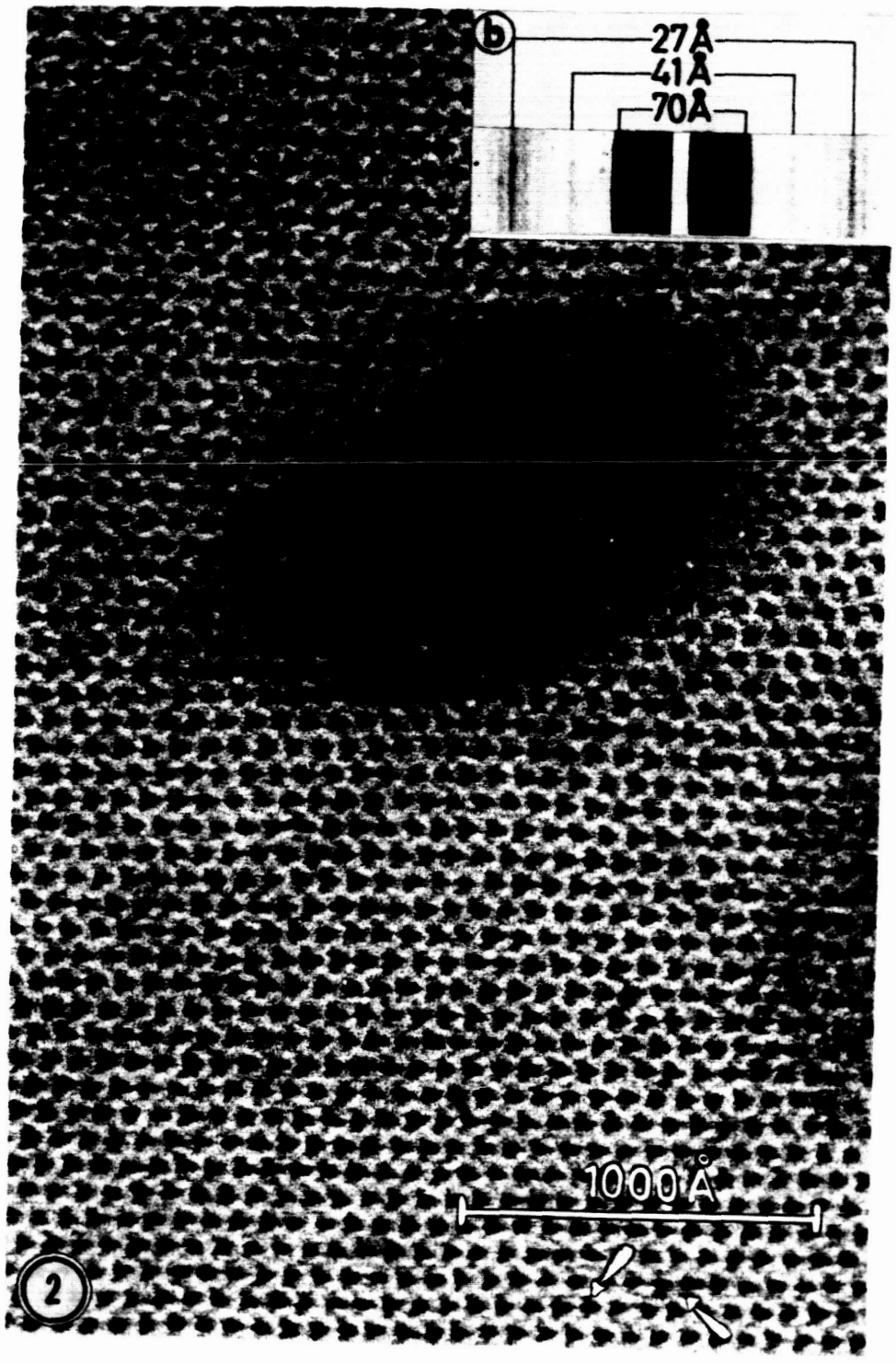
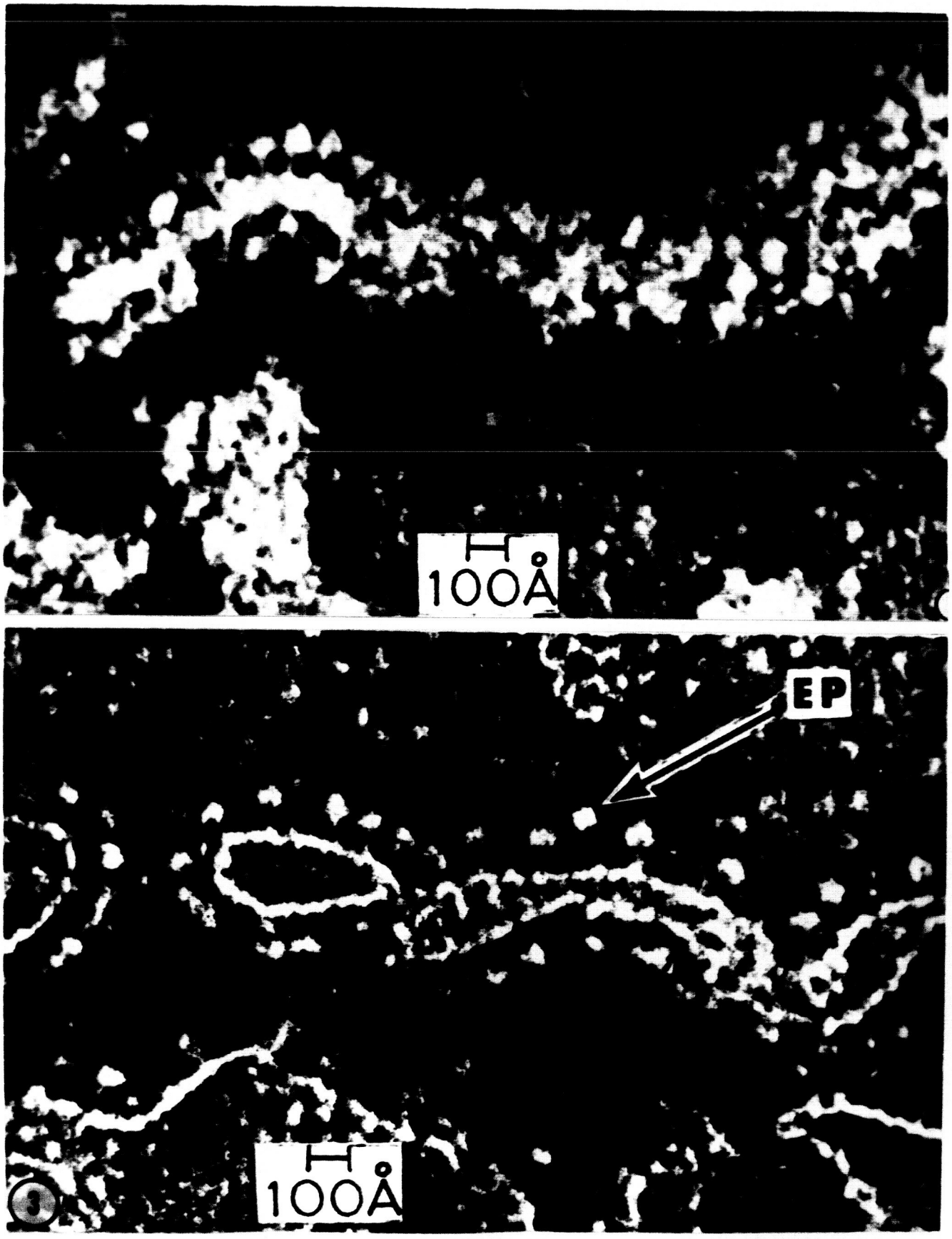
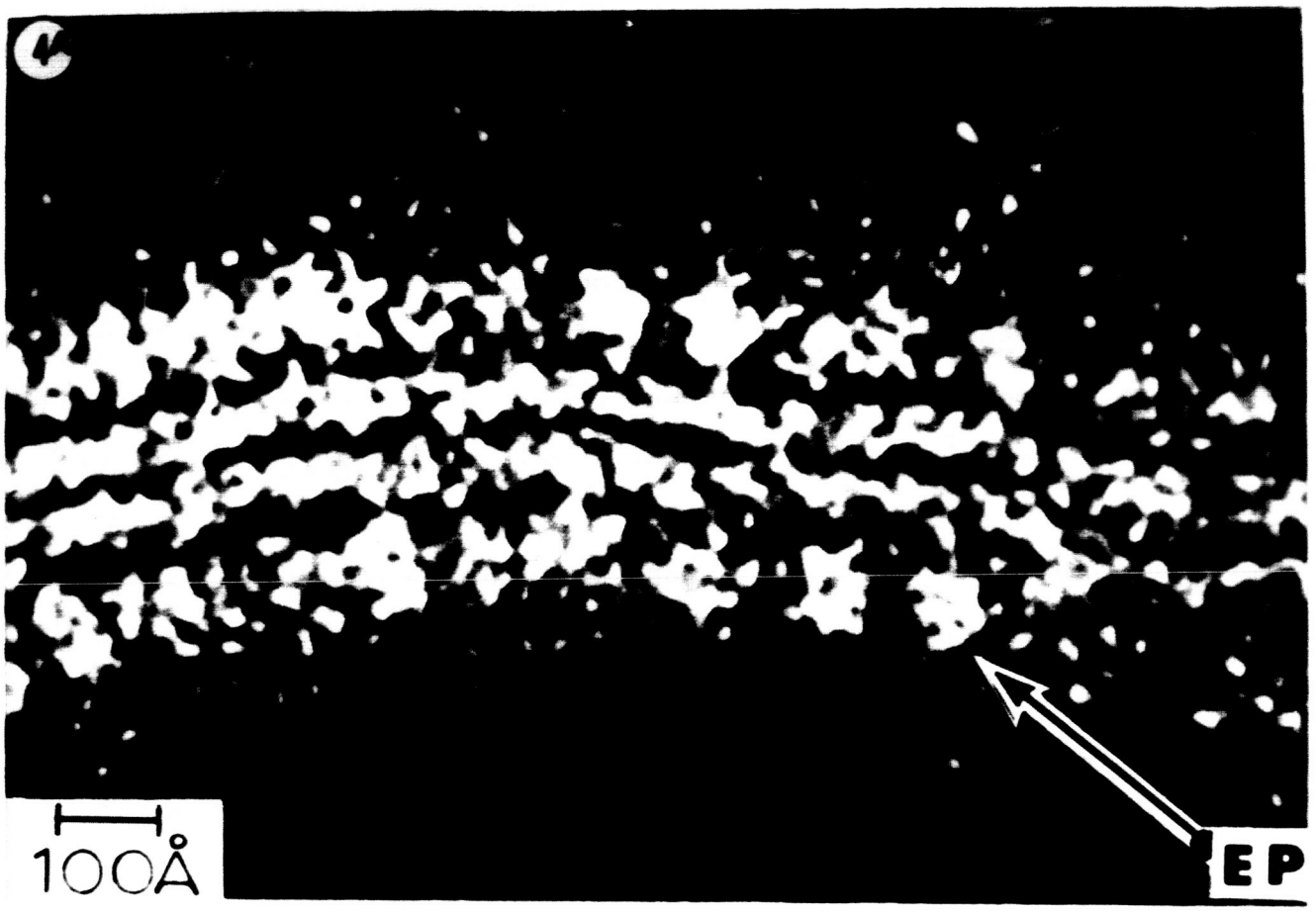
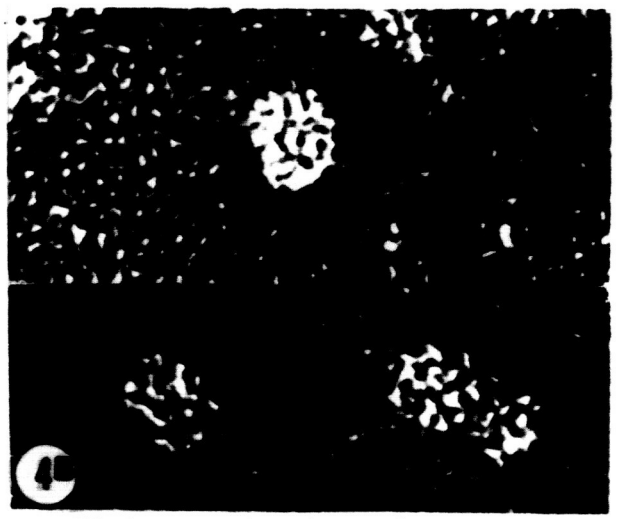

1
1000Å
b
178
89
59
44.5
36 Å

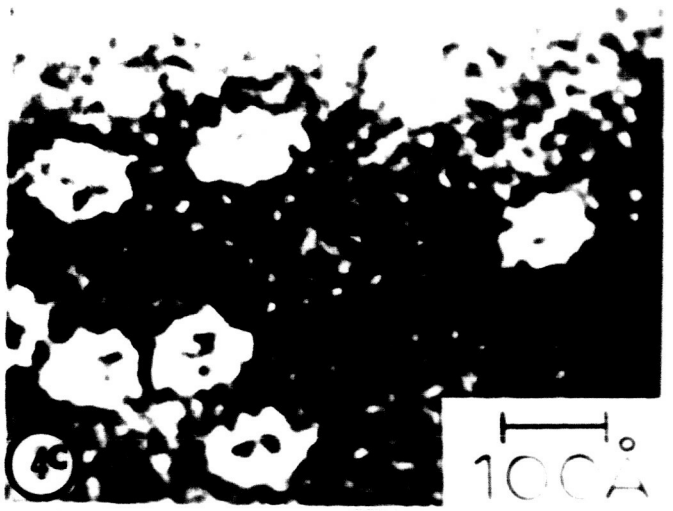
Plate 2




100Å
EP
100Å
3







High resolution electron micrographs of negatively-stained:

(a) Membrane segment (cristae) from isolated beef heart mitochondria with paired arrays of "elementary particles" "EP" (showing dense substructure) attached to central membrane layer.

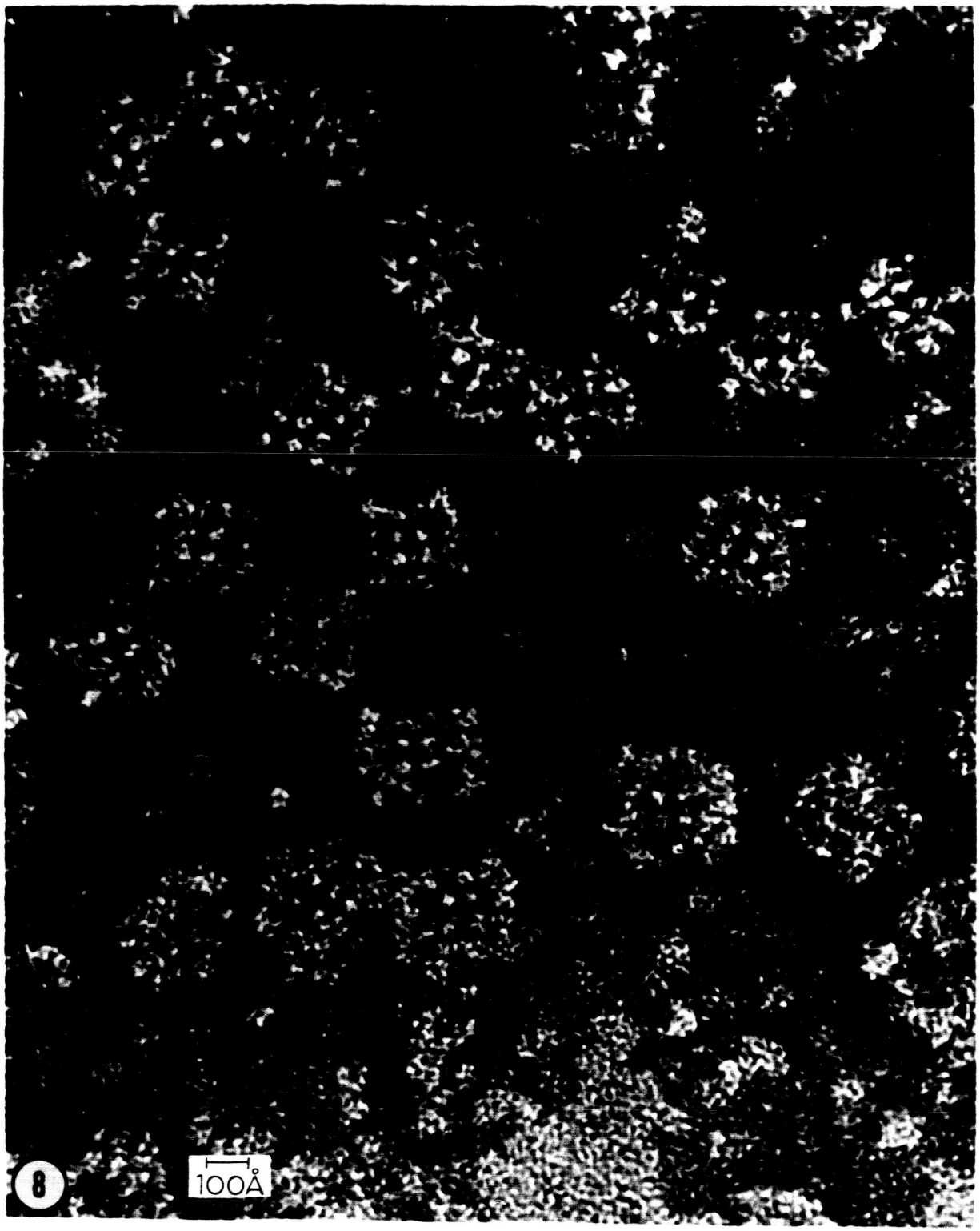
(b) Isolated particles that contain complete electron transfer chain.

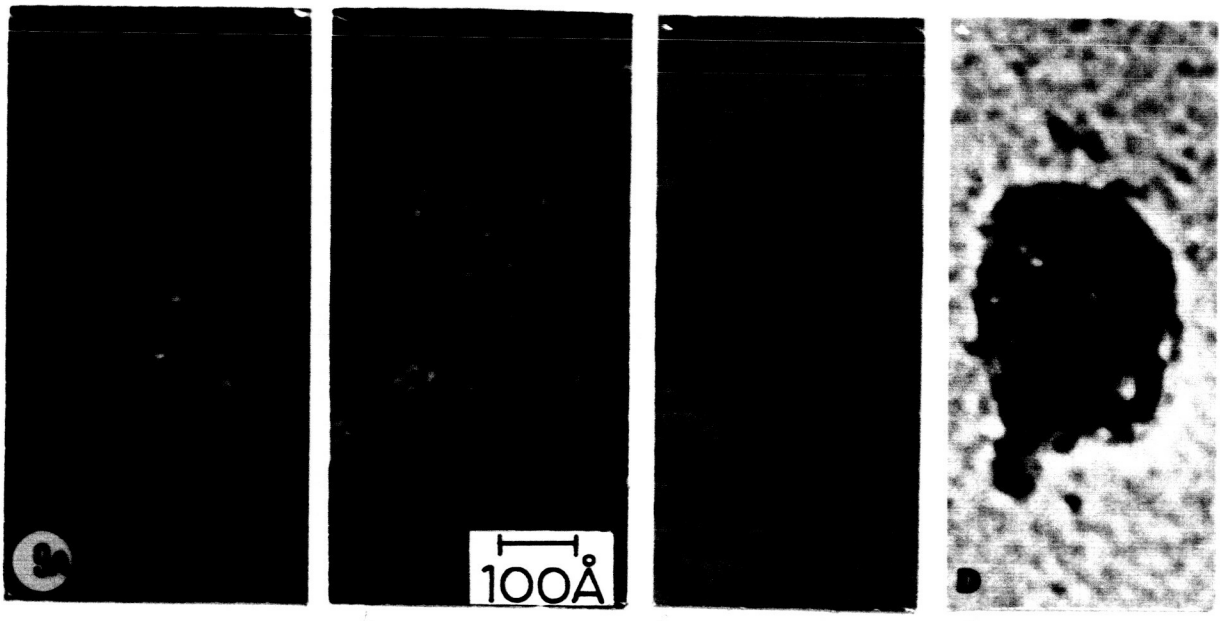
(c) Reconstituted electron transfer particles.

Magnifications: 1,300,000 X (indicated by scale of 100 Å)


6
100Å


7
100Å


8
100Å



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